Creosote P2 blend: 13-Week Inhalation Study in Rats
Creosote Council II. 1995. MRID No. 43600901

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Title: R. J. Hilaski; March 27, 1995; Thirteen week subchronic inhalation toxicity study on North American P2 Creosote CTM in rats.; International Research and Development Corp., Mattawan, MI 49071; Project No. 671-018; Creosote Council II; MRID # 43600901. Unpublished.

Performing Lab: IRDC, Mattawan, MI

Test Animal: One hundred seven (107) male and one hundred seven (107) female Crl:CD®BR VAF/PLUS® rats were received from Charles River Laboratories, Portage, Michigan July 27, 1993. Of these animals 80 males (209-256 g) and 80 females (140-175 g) were selected on the basis of appropriate body weight, absence of pre-existing ocular lesions and apparent good health. Five additional animals/sex were also selected for a pretest health screen to assure suitability. Animals were assigned at random to the control and treatment groups. Animals from each group assigned to the recovery interval were selected at random.

Test Material: The test material was received February 12, 1992 from H.A. Kremer and Associates (Ontario) Ltd. Bolton, Canada. The material was identified as North American P Creosote CTM and described as being black distilled coal tar with an odorous characteristic.

Dose: A two week range-finding inhalation study with P2 Creosote CTM (IRDC Study No. 671-017) was conducted on 4 groups of 10 rats/sex. Using the whole body exposure method, the animals were exposed to doses of 0, 15, 113 or 191 mg/m^3 at 6 hours/day for 5 days/week. No deaths occurred and no significant pharmacotoxic signs were noted. In addition to reduced terminal body weights (9.7-12.1%), increased absolute and relative liver weights of all animals of the mid and high dose groups were observed when compared with control values. Based on the results of this study it was recommended that for the 13 week study the highest dose of P2 should not exceed 100 mg/m^3.

The doses of P2 creosote selected for the definitive inhalation study were 0, 4.7, 48 or 102 mg/m^3 and these dose groups were referred to as Groups I to IV, respectively, in the study report.

Husbandry: All animals were identified by cage, group, sex and individually by a metal ear tag bearing the animal number. Animals were housed individually following an acclimation period of two weeks. They were maintained in environmentally controlled chambers with a 12 hour light/dark cycle. During acclimation, recovery, exposure and non-exposure periods temperature and humidity were within an acceptable range. The environmental conditions for this study were held more constant and within the parameters of the protocol than those observed for the P1/P13 creosote CTM test. During exposure days the relative humidity varied beyond the protocol limits on six of the 65 days treated. Water and diet (Rodent Chow® No. 5002, Purina Mills) were available *ad libitum*. All animals were housed in their respective chambers 24 hours per day during the 13 week exposure period except during cleaning. Chamber ventilation air was provided by an HVAC system and chamber exhaust air was filtered through a HEPA filter to remove particulates. Chamber airflow, temperature and relative humidity were recorded at one hour intervals during each days exposure and every three hours until midnight during non-exposure hours.

The animals were removed from the chambers prior to exposure so that the chambers could be cleaned of excreta and feed jars could be covered. The animals were removed for cleaning of chambers after exposure and feed was made available upon their return. Water was made available only during non-exposure times. Cage position was systematically rotated on a weekly basis.

Treatment Methods: Exposure was carried out in a 6 m^3 (6000 litre) whole-body exposure chamber. The aerosol atmospheres of the test material were generated by having warmed and stirred material metered at a constant rate to an atomizer operated with in-house compressed air. The air and test material produced a concentrated aerosol/vapour in a 4 litre glass atomization chamber. Additional air passing through another inlet purged the aerosol/vapour into the chamber inlet where the chamber-supply-air reduced the concentration to the desired level.

Exposure Parameters:

Atmosphere Generation Settings				
Group Number:	I	II	III	IV
Desired Conc. (mg/m^3):	0	5	50	100
Approx. Liquid Flow Rate (ml/min):	-	0.07	0.39	0.7
Purge Airflow (L/min):	-	10	40	40
Atomizer Pressure (psig):	-	10	30	30
Chamber Airflow (L/min):	1350	1350	1350	1350

Note: These were major difference in Group IV Flow rate compared to P1/P13 study. No weight changes of test material were reported in the study, thus limiting our ability to determine the nominal concentration and further the actual concentration. Comments from the registrant indicated that chamber concentrations were not affected.

Nominal Concentration: Nominal exposure concentrations were determined by taking the weight of the test material reservoirs before and after each exposure generation. The total volume of air passing through the chamber during the exposure was determined by taking an arithmetic mean of the flow rate measurements and multiplying by the exposure duration. Finally, the total amount of material used was divided by the total volume of air passed through the chamber during exposure to give a calculated nominal concentration in mg/m^3.

Actual Concentration: Actual concentrations were measured by standard gravimetric methods. Two samples were collected on 25 mm glass-fiber filter pads from each treatment group chamber during each six hour exposure. The sampling period was approximately two and one-half (2.5) hours or about half the treatment period so that two samples represent the concentration over the full day's exposure. Total volume sampled was measured with a dry gas meter. The difference in the pad pre- and post-sampling weights was calculated and divided by the sample volume.

Group No.	Sample Flow Rate (L/min)	Sample Duration (min)	Sample Volume (L):(ft^3)
II	5	150	750:27.0
III	1	150	150:5.3
IV	0.5	150	75:2.7

Aerosol Particle Size Determination: Determined once each exposure day for each group. The samples were collected using a cascade impactor operated at a flow rate of 28.3 L/min for an appropriate duration. Net weight on each filter pad was determined and computer analysis using cumulative percentages helped derive aerodynamic diameters smaller than the cutoff. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were generated by computer and calculated.

Chamber Distribution Evaluations: Prior to the first day of exposure the homogeneity of test material distribution was determined for each exposure chamber by comparing five locations to a reference location (routine sampling location). Homogeneity of the exposure appeared to be within acceptable (± 6-16 %) limits when sampled from five locations and compared with the reference location.

Chemical Characterization: Nine creosote components were determined using a compound specific gas chromatographic (GC) analytical method. Similar 25 mm glass-fiber filters used in the sampling of the aerosol atmosphere were collected and eluted with methylene chloride and an internal standard solution. The resultant solution and the bulk material were then analyzed using the sponsor supplied (GC) methods. Selected creosote vapours were also determined by GC using NIOSH method 1501. Samples were drawn from the exposure chamber, absorbed onto 1800/200 mg activated charcoal tubes, eluted with tetralin and carbon disulphide solution and analyzed by GC. Weekly samples of creosote were also collected and shipped to Geochemical and Environmental Research Group for analysis. (When the nine components were compared with those from the P1/P13 study differences were very minor).

Evaluation Parameters:

In-Life Examinations: Observations for mortality, morbidity or reaction to treatment were made daily. Thorough clinical examinations, body weight and feed consumption determinations were made weekly. Ophthalmoscopic examinations of the cornea, conjunctiva, sclera, iris and fundus were performed at pretest and prior to terminal or recovery sacrifice. Clinical laboratory tests were conducted on 5 animals/sex at pretest, 10 animals/sex/group at study termination and on all surviving animals at the end of the recovery period. Blood samples were obtained from the orbital sinus following an overnight fasting period.

Hematological parameters assessed: leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocytes, platelet count, differential leukocyte count.

Biochemical parameters assessed: concentrations of sodium, potassium, chloride, calcium, inorganic phosphorous, total bilirubin, urea nitrogen, creatinine, total protein, albumin, globulin, albumin/globulin ratio, serum cholesterol, glucose, and activities of alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferse (ALT), creatinine phosphokinase (CPK), ornithine carbamoyltransferase (OCT) , gamma glutamyltranspeptidase (GGT).

Necropsy: Any animals found dead during the course of the study were necropsied. At the terminal and recovery sacrifice all animals were euthanized by intraperitoneal injections of sodium pentobarbital. All animals were subjected to a complete postmortem examination. The following organ weights were determined: adrenals, brain, ovary/testis, heart, kidney, liver, lungs, mammary gland, thymus, thyroid/parathyroid.

Histopathological Examination: Representative sections of the following organs and tissues were collected from all Group 1 and Group IV animals designated for sacrifice after 13 weeks, as well as from any animals that died during the course of the study: adrenals, aorta, bone, bone marrow, bone marrow smear, brain, eye, optic nerve, GI tract, ovary/testis, heart, kidney, lacrimal gland, liver, lung, lymph nodes, mammary glands, nasal tissues, pancreas, pituitary, prostate and seminal vesicles, salivary gland, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, cervix, vagina and all gross lesions.

In addition, sections of the bone, eye, optic nerve, kidney, lung, nasal tissues, spleen, thyroid, heart and trachea were examined microscopically for all animals in Groups II and III at the 13-week terminal sacrifice and all animals in all groups at the recovery sacrifice. A four-step grading system of trace, mild, moderate and severe was used to define gradable lesions for comparison between dosage groups.

Statistics: Non-parametric analysis was conducted when the animal numbers in any one group was equal to or less than ten with the Kruskal-Wallis one-way analysis of variance followed by the Mann-Whitney U test where appropriate. On data where the number of animals were greater than ten and the measurements were at least an interval scale, parametric analysis was performed using Bartlett's chi-square test for homogeneity of variance followed by an analysis of variance and where appropriate by Dunnett's t-test. The level of rejection was at the five percent level in all cases.

RESULTS:

Exposure Conditions: In Table I, the mean dose concentration of the treated groups was within an acceptable degree of variability. The flow rate used for P2 aerosol generation was substantially lower, in all Groups, than that used in the P1/P13 generation. It was later indicated that this did not affect chamber concentration.

Table I Thirteen Week Mean Dose Concentrations

Group Number	Exposure Period (wks)	Times Daily Limit Exceeded	Desired Conc. (mg/m^3)	Actual Mean (Gravimetric) ($mg/m^3 \pm$ S.D.)	Nominal Mean ($mg/m^3 \pm$ S.D.)
II	13	19	5	4.7 ± 0.66	25 ± 2.3
III	13	3	50	48 ± 6.4	130 ± 8.8
IV	13	6	100	102 ± 7.0	283 ± 33.0

The aerosol size distribution along with the cumulative weight percent for particle size lower than 1.05 μm are seen in Table II below. Weekly MMAD values did not vary significantly and the GSD were similar in all cases. The MMAD was observed between acceptable values of 1 - 4 μm in size.

Table II Mean Particle Size Distribution and Cumulative Weight Percent

Group No.	No. of Days	Aerosol Size MMAD	GSD	Cumulative Weight Percent below 1.05 μm	Cumulative Weight Percent below 4.70 μm
II	65	2.9	1.91	6.88	78.5
III	65	2.4	1.85	9.03	80.3
IV	65	2.5	1.91	9.00	84.5

data from pages 211-212 of the report.

Aerosol analysis

The aerosol analysis revealed a low overall mean concentration of the nine components measured. As the dose increased the most affected components included: fluorene, fluorenthene, phenanthrene and to a small extent dibenzofuran. About 15 % of the test material readily vaporized under the experimental generation conditions and Table III identifies the common components. Components analyzed in the vapour phase consisted of the lighter more volatile compounds. Some components appeared to show a high degree of variability between monthly analysis for the respective doses. As an example, naphthalene was the component which appeared to have the widest variability where in groups II, III and IV concentrations ranged from 14.1 - 379 µg, 5.2 - 543 µg and 269 - 813 µg, respectively. While the report mentioned that both the analysed means and standard deviations were listed in Tables 5 and 6, only the mean values were present. A striking observation of the vapour analysis was that November values showed many vapour components drop to 0.0 in Groups II and III while values in Group IV remained relatively constant compared with previous months. No values were recorded for the month of August in Table 6 of the study.

Table III Vapour Components of Chamber Atmosphere

Component	Overall Group Mean (mg/volume of air sampled)		
	II	III	IV
Benzene	4.0	13.5	64.9
Toluene	28.2	46.3	203
Ethylbenzene	10.2	25.1	101
m + p xylene	22.7	56.8	219
o-xylene	9.6	23.6	93.4
cumene	0.0	1.98	7.9
Naphthalene	235	306	577
2-methylnaphthalene	46.5	48.1	85.3
1-methylnaphthalene	20.2	20.8	35.9
Total	376.1	542.0	1386.9

Volume of air varied for each group with an average of 480, 311 and 230 L of air for groups II, III and IV, respectively.

Mortality: Two animals (one female #60517 in Group II at day 34 and one male #60533 in Group III at day 66) were sacrificed *in extremis* during the exposure phase of the study. The cause of death of the Group III male was determined to be due to ulcers found in the non-glandular portion of the stomach. The cause of death of the Group II female could not be ascertained from the postmortem examination. In both cases, however, diffuse myeloid hyperplasia of the bone marrow and associated erythroid cell depletion were noted in the postmortem reports.

Clinical Signs: The most commonly noted observation in all groups was that of staining of various body areas although the discolouration was not identified. This observation was noted with greater frequency in males and females of treatment Groups III and IV and extended into the 6 week recovery period. Males and females of all treatment groups had scabs visible on various body areas. Other incidental observations noted in all groups included malocclusions and alopecia.

Body Weight: <u>Exposure Period</u> (weeks 1-13)

Table IV: Group Mean Body Weights (grams) in P2 Creosote-exposed Rats

Study week (n = 20)	Dose Group (mg/cubic m.)			
	0	4.7	48	102
Males				
week 1	280±14.2	276±12.8	270±11.5	259±16.6**
week 7	417±27.6	423±33.3	403±27.5	388±29.1**
week 13	462±43.9	478±45.3	447±35.8	437±33.6
Females				
week 1	182±8.8	177±9.3	174±9.9	165±18.7
week 7	254±15.6	247±14.5	241±16.9	241±15.9
week 13	284±23.7	269±20.9	262±21.6**	261±19.6**

data taken from page 73 and 75 of the report. *$p < 0.05$ or **$p < 0.01$ vs. control.

Males
Group mean body weight in male rats at the high dose of P2 creosote, as with P1/P13 creosote, was significantly decreased at the high dose by 7-8% in relation to control.. This decrement was evident throughout the study except at weeks 9,10 and 13. In female rats, group mean body weight was decreased by a similar percentage (6-10% vs control) throughout the study, except that in comparison to male rats, significant decreases at the high dose were not observed at weeks 2, 6, 8, and 9. Terminal mean body weights for males of Group II and III, respectively, were +3.5% and -3.2% of the control group weights and were not considered significantly different.

During the 13-week exposure period Group II, III and IV female mean body weights were significantly lower ($p<0.05$) than controls in 2/13, 7/13 and 9/13 weeks, respectively.

<u>Mean group body weight gain</u> In creosote treated male rats, weight gain for the 13 week study period was not significantly affected at any dose level. In female rats, weight gain was decreased in relation to control weight gain at all dose levels, but there was no dose-response (10%, 14%, and 6% at the low, mid, and high dose levels).
For Group II animals the 10% decrease in body weight gain was not considered toxicologically significant for two reasons. Firstly, mean body weights were only significantly different from control body weights during two consecutive weeks (10 and 11) of the entire exposure period and, during the first of these two weeks, their food consumption was found to be decreased by 17.5% for some unexplained reason as food consumption for weeks 9 and 11 was decreased only by 3.2 and 6.6 %, respectively. (This may have been due to some errors in the feeding protocol as females in Group III and IV and males in Group III showed significant decreases in their food consumption for week 10. See section on **Food Consumption**). Secondly, results of the range-finding study in which Group II animals received 15 mg/m^3 of the test material in air (cf. with 4.7 mg/m^3 in the present

study), the body weight gain for these females was only decreased by 8.7% and was not deemed to be toxicologically significant.

Recovery Period (weeks 14-19)
Following the six week recovery period Group IV animals gained weight rapidly as shown in the table below. Group mean body weights of all treated animals were comparable to control animal body weights.

Mean Body Weight Change

Exposure Weeks	Group II ♂	Group II ♀	Group III ♂	Group III ♀	Group IV ♂	Group IV ♀
1 - 13	-1.7	-14.0	-11.5	-18.4	-10.1	-12.6
14 - 19	+3.8	+1.9	-4.2	-0.1	-2.4	+7.2

1 - 13 exposure, 14 - 19 recovery

Food Consumption: Food consumption (mg/kg/day) is presented in the following table:

Group Mean Food Consumption (mg/kg/day) in P2 Creosote-exposed Rats

Study week (n = 20)	Dose Group (mg/cubic m.) 0	4.7	48	102
Males				
week 1	88.3±5.01	69.7±3.0**	67.8±3.13**	79.1±3.52**
week 7	59.7±9.92	60.5±3.68	60.6±2.99	63.8±4.26
week 13	48.5±7.34	47.9±2.78	48.7±2.89	51.0±3.51
Females				
week 1	98.5±8.57	81.4±8.85**	78.5±7.17**	83.3±11.09**
week 7	74.1±3.21	77.0±3.31	77.4±7.53	77.6±6.69

week 13	61.8±3.67	61.9±3.27	64.5±5.75	65.2±6.16

data taken from page 78 and 82 of the report. *$p < 0.05$ or **$p < 0.01$ vs. control.

Statistically significant decreases in food consumption were observed in male rats at week 1 of the study at all dose levels. These decreases ranged between 11-23% below control, but as shown, there was no definite dose-response for wek 1 food consumption. At week 2, significant decreases of 5% were observed at the 48 and 103 mg/cubic m dose levels. Beyond this time point, the only significant changes in food consumption were observed at the high dose on weeks 5 and 6 (where increases of 5 and 6% were observed), and at the low and mid dose on week 10, where a decrease of 7% was observed. On a g/animal basis, food consumption in male rats was decreased during weeks 1 and 2 in a similar manner as described above, and at the mid dose on weeks 10, 11, and 12, decreases of 9-10% were observed in relation to control.

In female rats, significant decreases in food consumption were observed during week 1 of the study. Compared to control, decreases of 18-21% were observed. Beyond this time, the only significant change noted on a g/kg basis was at week 10 for the low dose where a decrease of 11% was observed. On a g/animal basis, food consumption was observed to be significantly decreased (20-25%) at all dose levels during week 1 of the study. A significant decrease during week 10 was also observed at all dose levels (11-18%), but as during week 1, there was no dose-response (i.e food consumption affected similarly at all dose levels). At week 11, the high dose female test group showed decreased food consumption (9% decrease from control).

The observation that body weight Food consumption during the 6 week recovery period was comparable to that of control group animals.

Ophthalmology: No test article-related ophthalmoscopic abnormalities were detected in any of the treated animals. Observations that were considered representative of pathology expected for this group of animals considering age, sex and strain included conjunctivitis and chorioretinal hypoplasia.

Clinical Pathology:

Hematology

Terminal Examination (See table below) Dose-related effects noted on hematological parameters reached statistical significance in the high dose group only. High dose animals had decreased haemoglobin content (male and female), % hematocrit and erythrocyte counts (female) and increased reticulocyte count and increased incidence of poikilocytosis (male and female).

	Hematology Observations (N=10)							
	Group I		Group II		Group III		Group IV	
Parameter	♂	♀	♂	♀	♂	♀	♂	♀
Hemoglobin (g/dl)	15.4	15.0	15.3	14.6	14.8	14.1	**14.2**[2]	**13.6**[2]
Hematocrit (%)	44.3	41.7	42.4	41.0	40.5	38.5	39.6	**36.5**[2]
Erythrocyte ($x10^6/mm^3$)	7.75	6.99	7.40	6.92	7.01	6.56	6.96	**6.20**[1]
Reticulocyte (/100 RBC)	2.0	2.2	2.2	2.5	3.0	4.2	**4.2**[2]	**5.2**[2]
Mild Poikilocytosis	5/10	2/10	8/10	6/10	9/10	7/10	10/10	9/10

[1]Significantly different from control group; $p<0.05$
[2]Significantly different from control group; $p<0.01$

Platelet counts were decreased in all treated groups although counts were not statistically different from control group values (males 1033, 1001, 1016, 934 counts; females 1003, 974, 976, 931 counts in Groups I, II, III and IV, respectively).

Recovery Examination
Numbers of platelets were significantly decreased ($p<0.05$) in Group IV males at the end of the recovery period (1059, 1016, 995 and 894 counts in Groups I, II, III and IV, respectively). In females, platelet counts decreased when compared with control values, however, they did not attain statistical significance (1032, 932, 950 and 926 counts in Groups I, II, III and IV, respectively). The authors stated that the effects on platelet counts were not biologically significant. This would be a correct presumption as the variations noted are still within the normal range.

Mild poikilocytosis persisted through the recovery period and was observed in all treatment groups although the incidence was less in each group than that noted after the exposure period (2/10, 5/10, 4/10, 4/10 in males and 1/10, 0/10, 2/10, 2/10 in females of Group I, II, III and IV, respectively).

No other differences in hematological parameters from control group values were observed at the end of the recovery period for any treatment groups.

Serum Biochemistry
Terminal Examination (See table below) Statistically significant serum biochemistry effects included increased cholesterol levels in Group III (male) and Group IV (male and female) and increased total protein (male), globulin (male) and urea nitrogen (female) of treatment Group IV and decreased levels of alanine aminotransferase (ALT) in Group III and IV (male).
Increased cholesterol levels may be associated with decreased body weight or with observed histopathologic thyroid gland changes since serum cholesterol levels varies with thyroid gland activity.
The increase in levels of total proteins and globulins (males) and urea nitrogen (females) may be a response to the observed inflammatory changes in the trachea and nasal cavity epithelium (see microscopic pathology section). The decreased ALT levels of Group III and IV males was not of toxicological significance in this study.

Biochemical Parameter Observations (N=10)

Parameter	Group I ♂	Group I ♀	Group II ♂	Group II ♀	Group III ♂	Group III ♀	Group IV ♂	Group IV ♀
Total Protein (g/dl)	6.3	7.3	6.5	7.7	6.8	7.6	**7.0**[2]	7.8
Globulin (g/dl)	3.1	3.6	3.2	3.6	3.5	3.6	**3.6**[2]	3.6
Cholesterol (mg/dl)	52	72	64	76	**78**[2]	89	**77**[2]	**123**[2]
Urea Nitrogen (mg/dl)	11	12	10	13	11	14	11	**18**[2]
Alanine Aminotrans (U/L)	36	35	33	26	**26**[2]	28	**25**[2]	30

[1]Significantly different from control group; $p<0.05$
[2]Significantly different from control group; $p<0.01$

Recovery Examination No treatment group parameters were significantly different from control group values by the end of the recovery period.

Macroscopic Pathology:
Terminal Examination A diffuse grey discolouration of the lungs was observed in 10/10 (trace) Group III and 10/10 (trace-mild) Group IV male and female rats, all of which were related to the test article administration. All other observed macroscopic lesions were considered to be incidental in nature and unrelated to treatment.

Recovery Examination Treated animals showed a higher incidence of test article-related changes which included a tan discolouration of the lungs in all animals of both Group III and Group IV rats. The incidence and severity of these changes after the recovery period were lessened in comparison with those observed after the exposure period. All other macroscopic lesions were considered to be unrelated to treatment.

Organ Weights:
Terminal Examination (See table below)
Liver A test article-related increase in the absolute liver weight, liver/body weight ratio, liver/brain weight ratio in Group III and IV male and female rats was observed. However, no corresponding histopathologic hepatic changes were noted.

Kidney Kidney/body weight ratios were significantly increased in Group III and IV males and Group II, III and IV females. Corresponding kidney/brain weight ratios were not statistically significantly different from controls in any of the treatment groups, however, suggesting the changes in kidney/body weight ratios were a reflection of the reduction in the terminal body weights of these animals.

Thyroid/Parathyroid Significantly increased in the thyroid/parathyroid/body weight ratio was seen in Group IV females. Absolute weight and relative/brain weight were also increased but not statistically significant from control animals. Treatment-related histopathological changes in the thyroid follicular epithelial cells (trace-mild hypertrophy) observed in both males and females could account for the organ weight changes.

Numerous other significant increases in organ/body weight ratios were observed in females of Group III and IV. These included, heart/body weight and brain/body weight. These increased ratios are considered a result of decreased body weights in these groups, as are the statistically significant effects in organ/body weight ratios of Group II animals.

Recovery Examination
Liver The liver/body weight ratio was increased only in males of Group III and IV but no corresponding histopathological hepatic effects were noted.

Lung/trachea The lung/trachea/body weight ratio was significantly increased in Group IV males along with corresponding histopathological observations in the lungs.

Thyroid/Parathyroid Increased thyroid/parathyroid weights and ratios were observed at the end of the recovery period in both males and females of Group IV.

	Mean Organ Weight Values							
	Group I		Group II		Group III		Group IV	
Exposure Examination	♂	♀	♂	♀	♂	♀	♂	♀
Heart (g)	1.35	0.89	1.28	0.88	1.35	0.91	1.35	0.91
Heart/Body Wt. (%x10)	3.24	3.4	3.02	3.67	3.30	3.81	3.38	**3.96**[2]
Brain (g)	2.00	1.84	2.01	1.86	1.99	1.82	1.94	1.84
Brain/Body Wt. (%x10)	4.79	7.09	4.74	7.86	4.86	7.59	4.85	**8.04**[1]
Liver (g)	11.07	6.97	11.86	6.97	13.36	**7.96**[1]	**14.09**[2]	**8.61**[2]
Liver/Body Wt. (%)	2.60	2.66	2.78	**2.92**[1]	**3.25**[2]	**3.31**[2]	**3.51**[2]	**3.72**[2]
Liver/Brain Wt. (%x10^{-2})	5.56	3.79	5.90	3.75	6.72	**4.38**[1]	**7.25**[2]	**4.69**[1]

Exposure Examination	Mean Organ Weight Values							
	Group I		Group II		Group III		Group IV	
	♂	♀	♂	♀	♂	♀	♂	♀
Kidney (g)	2.90	1.80	3.03	1.83	3.02	1.90	3.03	1.97
Kidney/Body Wt. (%x10)	6.88	6.93	7.13	**7.68**[2]	**7.36**[1]	**7.90**[1]	**7.57**[1]	**8.53**[2]
Kidney/Brain WT. ($\%x10^{-2}$)	1.46	0.98	1.50	0.98	1.52	1.04	1.56	1.07
Lung/Trachea (g)	1.67	1.30	1.58	1.22	1.69	1.30	1.67	1.27
Lung/Trachea/Body Wt. (%x10)	3.99	4.97	3.72	5.12	4.11	5.44	4.17	**5.51**[1]
Lung/Trachea/Brain Wt. ($\%x10^{-1}$)	8.36	7.04	7.86	6.55	8.49	7.18	8.63	6.90
Thyroid/Parathyroid (mg)	20	16	19	15	21	16	21	17
Thyroid/Parat./Body Wt. ($\%x10^{3}$)	4.84	6.14	4.47	6.35	5.01	6.48	5.28	**7.55**[2]
Thyroid/Parat./Brain Wt. (%x10)	10.1	8.7	9.5	8.2	10.3	8.6	10.9	9.5
Recovery Examination								
Liver (g)	12.37	7.43	13.52	8.04	13.51	7.60	13.57	8.36
Liver/Body Wt. (%)	2.59	2.70	2.65	2.83	**2.89**[2]	2.88	**2.93**[2]	2.99
Liver/Brain Wt. ($\%x10^{-2}$)	6.16	4.01	6.67	4.27	6.82	4.19	6.79	4.50
Lung/Trachea (g)	1.76	1.39	1.90	1.44	1.86	1.29	1.93	1.37
Lung/Trach./Body Wt. (%x10)	3.68	5.09	3.73	5.10	3.99	4.91	**4.18**[1]	4.91
Lung/Trachea/Brain Wt. ($\%x10^{-1}$)	8.71	7.52	9.35	7.68	9.35	7.09	9.67	7.37
Thyroid/Parathyroid (mg)	18	12	22	13	21	14	**23**[1]	**19**[2]
Thyroid/Parat./Body Wt. ($\%x10^{3}$)	3.91	4.46	4.28	4.65	4.39	5.46	**4.95**[2]	**6.84**[2]
Thyroid/Parat./Brain Wt. (%x10)	9.2	6.6	10.8	7.0	10.4	7.9	11.5	**10.3**[2]

[1]Significantly different from control group; $p<0.05$
[2]Significantly different from control group; $p<0.01$

Microscopic Pathology:
Terminal Examination

Treatment-related effects were observed in the lungs, nasal cavity epithelium and thyroid glands of male and female rats.

<u>Lungs</u>

Test article-related effects consisted of one to three small, black or brown foci of granular pigment within the cytoplasm of alveolar macrophages uniformly distributed throughout the alveolar spaces of the lung. Pigmentation was observed at an incidence of 0, 6, 11 and 10 in males and 0, 7, 10 and 10 in females respectively in Groups I, II, III and IV (N=10).

<u>Nasal tissues</u>

(See table below) All four levels of the nasal tissues were observed to have changes in the olfactory epithelium in all groups. The major changes were seen in the nasal tissue A (the most anterior section). The incidence and severity of nasal lesions decreased progressively from nasal tissue A to nasal tissue D. Test-article related lesions consisted of chronic inflammation, dilated/cystic glands, epithelial hyperplasia, squamous metaplasia and lymphoid hyperplasia. Incidence was highest in Group IV. Chronic inflammation was present in 4/10 male and 1/10 female control animals. The incidence of hyperplasia of the lymphoid tissue in nasal tissue C and D was highest in Group IV.

Test Article Related Nasal Tissue Lesions
Incidence in Nasal Tissue Sections A, B, C and D

Group	I		II		III		IV	
Sex	M	F	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10	10	10
Nasal Tissue A	4	1	7	7	8	5	10	9
Nasal Tissue B	1	1	1				1	1
Nasal Tissue C	3	3	2	1	2		9	9
Nasal Tissue D							5	3

Thyroid Follicular epithelial cell hypertrophy of the thyroid gland was observed in all male groups including the control (7/10, 9/10, 10/10 and 10/10, respectively) and in 10/10 females of Group IV. No changes were observed in low and mid dose females. Severity of the diffuse hypertrophy in the high dose females was 5-trace and 5-mild. The highest severity rating of mild in the males was observed in 0, 1, 5 and 9 animals respectively. As dose increased male animals showed an increase in severity from trace to mild. A gradual increased effect was noted in male groups while an effect was evident in Group IV females. Comments from the author suggest these manifestations were test article-related in the females but were a physiologic response unrelated to treatment in the males. No explanation was presented for the physiological response.

Other histopathological changes in various organs included the following:
Trachea Chronic tracheal inflammation was observed at a low incidence in males (0/10, 2/10, 1/10 and 0/10) and females (0/10, 1/10, 4/10 and 0/10) of the two mid dose groups. Lesion incidence was low and was thought to be related to the dose method and not the test article. Interpretation was based upon recovery sacrifice data in which similar incidence of inflammation was observed (5/10, 1/10, 1/10 and 5/10 for male; 4/10, 2/10, 1/10 and 6/10 for female, respectively).

Kidney Histopathological changes in the kidney of Group IV animals included cast formation, lymphocytic infiltration, basophilic cortical tubules and tubulointerstitial nephritis. Similar effects were seen in Group I females along with tubular dilatation, tubular microconcretions and dilatation of the renal pelvis. The occurrence of such histopathological changes in male and female rats is not an uncommon finding in animals of this age. Observations were within background incidence at the time of recovery sacrifice.

Recovery Examination
Lungs
As in the terminal sacrifice there was a wide dispersion of pigment granules within alveolar macrophages throughout the lobes of the lung. Pigmentation was observed in 0, 2, 7 and 9 in males and 0, 0, 8 and 10 in females, respectively, in Groups I, II, III and IV (n=10). No inflammatory response to pigmentation was noted.

Nasal tissues
(See table below) Lesions of the nasal tissues lessened in incidence and severity during the recovery period. Chronic inflammation was the major nasal tissue lesion observed in terminal sacrifice animals and its incidence at the end of the recovery period was lower.

Test Article Related Nasal Tissue Lesions
Incidence in Nasal Tissue Sections A, B, C and D

Group	I	II	III	IV

Sex	M	F	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10	10	10
Nasal Tissue A	1	1	1		2		6	5
Nasal Tissue B	2	1	2		3	3	6	7
Nasal Tissue C								1
Nasal Tissue D								1

Thyroid

Hypertrophy of thyroid follicular epithelial cells with a subsequent reduction in the quantity of colloid was observed in both male and female rats. This effect appeared to be increased in incidence when compared with that observed in low and mid dose females at terminal sacrifice. Incidence was 0/10, 2/10, 5/10 and 4/10 for Groups I, II, III and IV females, respectively. Incidence in males (7/10, 8/10, 7/10 and 9/10 for Groups I, II, III and IV, respectively) was similar to that observed in the terminal sacrifice animals. However, incidence severity in males was increased throughout the dose groups (grade of mild in 0, 2, 5 and 8, respectively). As noted in the P1/P13 study, a difference of interpretive opinions between the two pathologists that reviewed the slides was evident. On this occasion the peer review pathologist reduced the study pathologist's incidence score either from moderate to mild or from mild to trace. Clearly this type of inconsistency makes it difficult to assess the true effect of creosote on the thyroid. The final outcome of the NOEL will not be affected by including the thyroid data. Therefore, inclusion of thyroid data in the hazard assessment of creosote P2 is disregarded. However, it should be noted that should any long term studies be conducted, this parameter should be closely monitored and scrutinized.

A peer review of the unaudited draft of histopathology data was conducted by Suzanne Botts, D.V.M., Ph.D., A.C.V.P. Diplomate of Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina.

Author's Conclusion:

Exposing groups of male and female rats to aerosol concentrations of 4.7, 48 and 102 mg/m^3 of North American P2 creosote CTM for 13 weeks (6 hours/day, 5 days/week) produced several treatment related changes, primarily at the two highest exposure levels. By the end of the recovery sacrifice (6 week post exposure) most exposure-related effects were no longer evident. Long term toxicological significance of treatment related effects at these two doses cannot be determined from this study.

No observable effects were produced in rats following exposure to P2 creosote CTM at a concentration of 4.7 mg/m^3 (0.0047 mg/L in air) for 13 weeks.

Evaluator's Comments:

The study protocol and report are acceptable. The following treatment-related changes observed in the mid dose (48 mg/m^3) and high dose (102 mg/m^3) animals (Groups III and IV, respectively) at the end of the exposure period were considered relevant in establishing a NOAEL at the lowest dose of 4.7 mg/m^3 for P2 creosote CTM.

1. Decreased terminal mean body weight and mean body weight gain (Group III & IV - **m/f**).
2. Changes in hematological parameters indicating an anaemic state of health (decreases in erythrocyte counts, % hemoglobin and hematocrit), an effect on erythropoiesis (increased reticulocyte counts, altered erythrocyte morphology as indicated by the presence of mild poikilocytosis).
3. Changes in serum biochemistry parameters (increased cholesterol levels) (Group III - **m**, Group IV - **m/f**).
4. Increased incidence of thyroid follicular epithelial cell hypertrophy (Group IV - **f**). Absolute and relative thyroid weights of Group IV animals actually increased after the recovery period (**m/f**).

5. Increased incidence and severity of lesions of the nasal cavity epithelium (chronic inflammation). Effects were lessened by the end of the recovery period (Group III & IV - **m/f**).
6. Significant increases in absolute and relative liver weights although there were no concurrent histopathological changes (Group III & IV - **m/f**). Resolved by the end of the recovery period except for liver/body weight ratio of Group III & IV - **m**).
7. Increased lung/trachea/body weight ratios, macroscopic grey discolouration of the lung tissue and microscopic presence of black pigment granules within alveolar macrophages (Group IV - **m/f**).

Animals of the low dose group (4.7 mg/m^3, Group II) also had a slight increased incidence of trace thyroid follicular epithelial cell hypertrophy (males), mild poikilocytosis (males) and low incidences of nasal epithelial lesions (resolved by the end of the recovery period). Thyroid follicular cell hypertrophy persisted throughout the recovery period. However, due to the discrepancies noted in the grading of the thyroid effects, this parameter was not used in the selection of a NOEL and was disregarded from the hazard assessment.

After the 6 week recovery period terminal body weights of all treated groups were comparable with those of control group animals. Histological changes in the thyroid were still evident (slightly decreased incidence in all male groups and high dose females but slightly increased incidence in low and mid dose females). The issue of differences in interpretive opinion between the two reviewing pathologists is a confounding factor. However, the severity of the follicular cell hypertrophy still remains border line and of questionable toxicological significance. Treatment-related changes in the haematological parameters were largely resolved by the end of the recovery period (except for the observation of poikilocytosis). The long term toxicological significance of these findings (anaemia and alterations of haemopoiesis) cannot be determined from this study, particularly as the treatment protocol (5 days/week) allows for a 2-day recovery period throughout the exposure period.

Synopsis:

In a 13-week inhalation toxicity study (MRID # 43600901), 20 Sprague-Dawley rats/sex/group were treated for 5 days/week, 6 hours/day with P2 Creosote CTM via whole body exposure at doses of 0, 4.7, 48 or 102 mg/m^3 (0, 0.005, 0.048 or 0.102 mg/L) in air measured gravimetrically. The aerosol size MMAD was between 2.4 and 2.9 microns with a geometric standard deviation between 1.85 and 1.91. Subsequent to the exposure period 10 rats/sex/group were allowed to recover from treatment for 6 weeks.

During the exposure period, two animals (low dose female; mid dose male) were sacrificed in extremis and the cause of morbidity was not related to treatment. Significant treatment-related findings in mid and high dose animals included decreased terminal body weight and body weight gain (m/f), altered hematological parameters (decreased hemoglobin content, hematocrit, erythrocyte and platelet counts; increased reticulocyte counts and mild poikilocytosis, m/f) and biochemical parameters (increased serum cholesterol levels, m/f). In both sexes macroscopic discolouration of the lungs persisted through the recovery period and correlated with the presence of black pigment granules within alveolar macrophages. Both sexes showed increased absolute and relative liver and thyroid weights and increased lung/trachea/body weight ratios. Absolute and relative thyroid weights of high dose animals actually increased after the recovery period. An increased incidence of lesions of the nasal cavity epithelium (chronic inflammation) was noted following treatment (all treatment groups, m/f) but appeared to lessen in incidence and severity during the recovery period (mainly the high dose group, m/f). During exposure an increased incidence of thyroid follicular epithelial cell hypertrophy occurred in all male groups including control and in the high dose female group. At recovery the male incidence remained similar to that observed at exposure while the incidence in females of the high dose group had declined. The incidence of thyroid follicular cell hypertrophy was slightly increased in low and mid dose females after the recovery period. Slightly increased incidence of mild poikilocytosis was observed in all treatment groups (m/f) including the low dose group and control, which persisted through the recovery period. Low dose animals exhibited lesions of the nasal cavity epithelium which had resolved after the recovery period. Based on the results of this study, the systemic LOAEL is 48 mg/m^3 , based on decreased body weight and weight gain, altered hematology ad clinical chemistry, increased absolute and relative weight of the liver ad thyroid, and increased incidence of lesions of the nasal cavity. The systemic NOAEL is set at 4.7 mg/m^3 (0.0047 mg/L) for P2 Creosote CTM in rats.

This study is classified as **acceptable** (guideline) and satisfies the guideline requirement (OPPTS 870.3465; OPP 82-4) for a subchronic inhalation toxicity study in rats for P2 creosote.